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Increased Markers of Cardiac Vagal Activity in Leucine-Rich Repeat Kinase 2-Associated Parkinson's Disease

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Running title Vagal markers in LRRK2-PD

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Abstract

Background Cardiac autonomic dysfunction manifests as reduced heart rate variability (HRV) in idiopathic Parkinson's disease (PD), but no significant reduction has been found in PD patients who carry the *LRRK2* mutation. Novel HRV features have not been investigated in these individuals. We aimed to assess cardiac autonomic modulation through standard and novel approaches to HRV analysis in individuals who carry the *LRRK2* G2019S mutation.

Methods Short-term electrocardiograms were recorded in 14 LRRK2-associated PD patients, 25 LRRK2-non-manifesting carriers, 32 related non-carriers, 20 idiopathic PD patients, and 27 healthy controls. HRV measures were compared using regression modeling, controlling for age, sex, mean heart rate, and disease duration. Discriminant analysis highlighted the feature combination that best distinguished LRRK2-associated PD from controls.

Results Beat-to-beat and global HRV measures were significantly increased in LRRK2-associated PD patients compared to controls (*e.g.*, deceleration capacity of heart rate: $p=0.006$) and idiopathic PD patients (*e.g.*, 8^{th} standardized moment of the heartbeat interval distribution: $p=0.0003$), respectively. LRRK2-associated PD patients also showed a significantly increased irregularity of heart rate dynamics, as quantified by Rényi entropy, when compared to controls ($p=0.002$) and idiopathic PD patients ($p=0.0004$). Ordinal pattern statistics permitted the classification of LRRK2-associated PD individuals with 93% sensitivity and 93% specificity. Consistent results were found in a subgroup of LRRK2-non-manifesting carriers when compared to controls.

Conclusions Increased beat-to-beat HRV in *LRRK2* G2019S mutation carriers compared with controls and idiopathic PD patients may indicate an augmented cardiac autonomic cholinergic activity, suggesting an early impairment of central vagal feedback loops in LRRK2-associated PD.

Keywords Autonomic dysfunction, heart rate variability, Parkinson's disease, LRRK2, deceleration capacity of heart rate, Rényi entropy

Parkinson's disease (PD) is a progressive multisystem degenerative process, involving motor and non-motor dysfunction associated with multiple neuroanatomical areas, neurotransmitters, and protein aggregates (Kalia & Lang, 2015). Symptoms and signs of autonomic dysfunction are common in idiopathic PD (iPD) and cardiac dysautonomia has been demonstrated by several measures from autonomic reflex tests to heart rate variability (HRV) analysis, all of which have consistently revealed a decreased HRV in iPD (Kallio et al., 2000; Maetzler et al., 2015; Rodriguez et al., 1996; Turkka et al., 1987). However, the effect of *LRRK2* mutations, the most common monogenic cause of PD (Healy et al., 2008), on autonomic function is still debated.

The most common mutation in the gene encoding leucine-rich repeat kinase 2 (LRRK2) results in a G2019S amino acid substitution, which increases the kinase activity of the protein (West et al., 2005). Symptoms of dysautonomia are frequent in LRRK2-associated PD (LRRK2-PD) (Tijero et al., 2013), although differences in non-motor symptoms have been found between LRRK2-PD and iPD patients. Some of us have previously found no significant alterations in cardiac autonomic modulation in *LRRK2* G2019S (NM_198578.3 (*LRRK2*): c.6055G>A, p.Gly2019Ser) mutation carriers, as assessed by traditional time and frequency domain HRV analysis (Visanji et al., 2017), although others have indicated significant modifications in some frequency domain measures (Solla, 2013). Thus, the involvement and timing of cardiac autonomic alterations over the course of LRRK2-PD remain unclear and the extent of dysautonomia is not fully understood.

This study aimed to assess cardiac autonomic modulation through standard and novel approaches to HRV analysis in individuals who carry the *LRRK2* G2019S mutation. The new approaches quantify the complex, non-stationary dynamics of heart rate (HR) and may therefore provide clinically relevant information that cannot be captured by traditional methods. Early recognition of autonomic impairment would potentially allow timely therapeutic intervention and could positively impact disease course, thereby improving patient quality of life and decreasing the social cost of PD. Furthermore, early biomarkers of prodromal PD are needed in preparation for the eventual application of disease-modifying therapies for LRRK2-PD.

Methods

Subjects

We studied 14 LRRK2-PD patients, 25 LRRK2-non-manifesting carriers (LRRK2-NMC), 32 related non-carriers (RNC) (non-manifesting family members without the *LRRK2* mutation), 20 iPD patients, and 27 unrelated healthy controls. Probands with *LRRK2* p.G2019S mutations, iPD patients and healthy individuals (without neurologic disease or family history of PD) were recruited at the Toronto Western Hospital (Ontario, Canada) and the Parkinson's Institute (California, USA). Family members of participants with the *LRRK2* mutation and iPD were also invited to participate. The presence or absence of p.G2019S was evaluated in all participants as previously described (Paisan-Ruiz et al., 2005). Subjects with iPD were defined as individuals with PD, according to clinical diagnosis by a movement disorder specialist, in the absence of a family history of the disease in first- or second-degree relatives. The study protocol was approved by the University Health Network Research Ethics Board (Toronto) and El Camino Hospital Institutional Review Board (Parkinson's Institute) and all participants provided written informed consent.

Clinical evaluation

Clinical evaluation included a neurological examination, standardized videotaping of the neurological examination, the Unified Parkinson's Disease Rating Scale part 3 (UPDRSIII), and the Scales for Outcomes in PD-Autonomic (SCOPA-AUT). Individuals taking anti-cholinergics, sympathetic agonists, or sympathetic antagonists or with evidence of thyroid dysregulation or diabetes were excluded from the study. Assessments were performed by experienced movement disorders clinicians blinded to genetic status as described previously (Marras et al., 2011). All participants with PD met UK Parkinson's Disease Society Brain Bank Clinical Diagnostic Criteria (Hughes et al., 1992).

Electrocardiographic recording and heartbeat intervals

Following five minutes of inactivity in a supine position, 7-minute resting 4-lead electrocardiograms (EKGs) (aV_R, aV_L, N, aV_F) were collected during daylight hours in a non-fasting state, and digitized at 500 Hz using a laptop-based cardio-card EKG system (Nasiff Associates, Inc., Central Square, New York). Normal-to-normal (NN) cardiac interbeat intervals were extracted from the EKG recording using Physionet WAVE v6.11 (www.physionet.org) in a Unix environment. The EKGs were manually checked for ectopic beats and regions of noise that were manually removed, following the application of an automated algorithm for obtaining NN interval data (Machado et al., 2000).

HRV analysis

Sequences of 300 NN intervals were analyzed, unless otherwise stated, using traditional and novel HRV methods (Supplementary Fig. 1). See Electronic Supplementary Material for further details.

Time domain methods. These measures included the standard deviation of the NN intervals (SDNN), the width of NN interval distribution (W, difference between the longest and shortest NN intervals), the coefficient of variation of the NN intervals (CV), the square root of the mean of the sum of the squares of differences between adjacent NN intervals (rMSSD), the first order autocorrelation coefficient (r_1), the autonomic stress index (ASI) (see Electronic Supplementary Material), and the standardized central moments of order m=3–9 of NN interval distribution.

Frequency domain methods. The power spectral density was calculated over NN interval sequences of 215 seconds for the low frequency band (LF) (0.04–0.15 Hz), the high frequency band (HF) (0.15–0.4 Hz), and the total spectral power band (TP) (see Electronic Supplementary Material). The LF/HF ratio, was also determined.

Information domain methods. The irregularity of NN intervals was determined by Shannon entropy (ShE), Rényi entropy (RE) and permutation entropy (PE), each of which distinguishes random from regular HR changes (Bandt & Pompe, 2002; Cornforth et al., 2014). ShE considers the probability of any NN value to appear in the data sequence. RE generalizes ShE to include measures at different scales (order α), and considers the probability of NN sequences of different length (λ) to appear in the HR signal. PE considers the probability of ordinal patterns (P) of different length (λ) occurring over different time scales (τ) of the HR signal.

Phase-rectified signal averaging (PRSA). The PRSA algorithm is based on averaging NN data segments around NN intervals previously defined as anchors (events that trigger particular HR changes), to quantify the average deceleration and acceleration capacity of HR (DC and AC, respectively) (Bauer et al., 2006).

Poincaré plot features. Additionally, we examined the standard deviation along the identity line (SD2) of an ellipse fitted to the scatterplot of each NN interval *vs.* the next, the standard deviation perpendicular to the ellipse identity line (SD1), and the SD2/SD1 ratio.

Statistical analysis

Statistical analyses were performed using STATISTICA software (StatSoft, Inc., Tulsa, Oklahoma). Continuous variables were assessed for normality by a Kolmogorov-Smirnov test, and were logarithmically transformed (Log) to adjust for skewness, except for the HRV feature r_1 which was transformed as: $0.5*\text{Log}((1+r_1)/(1-r_1))$. Group differences in HRV were assessed using multiple linear regression analysis, adjusted for age, sex, and mean HR. The LRRK2-PD *vs.* iPD contrast was also adjusted for disease duration. PE contrasts between age- and sex-matched groups were assessed using a Mann-Whitney *U* test, whereas a t-test was applied for contrasting the remaining continuous variables. Sex differences between groups were assessed using the Chi-Square test. Differences in the distribution of variables were assessed through a Kolmogorov-Smirnov test. Pearson r or Spearman R correlation coefficients were also determined.

Linear discriminant analysis was performed to identify the feature combination with the best discriminative power between groups, following a forward stepwise variable selection

procedure. Candidate features were extracted from a randomly-selected training sample, where the discriminant models were also estimated. The predictive accuracy of the classification functions was assessed in the remaining test sample with no overlap of cases. Participants in both training and test subsamples were age- and sex-matched. HRV values were standardized (Z-scores) considering the mean value adjusted for age, sex, and HR through multiple regression analysis, for those features affected by these confounders. Statistical significance was set at 0.05 and adjusted for multiple comparisons.

Results

Participant clinical and demographic characteristics

LRRK2-PD and iPD patients were of similar ages, while LRRK2-NMC individuals were significantly younger (Table 1). Disease duration was significantly longer in LRRK2-PD than in the iPD group, however no significant differences in the severity of motor signs (UPDRSIII) were found. Symptoms of autonomic dysfunction (SCOPA-AUT) were significantly more frequent in LRRK2-PD patients compared to controls, although no significant differences were found in the cardiovascular subscale. Information regarding orthostatic hypotension and L-dopa equivalent daily dose was not available for all patients.

Associations between HRV measures and clinical characteristics

HRV was not associated with disease duration in any of the PD groups. Among the iPD patients, only the global HRV measures (SDNN, W, CV, ASI, TP, and ShE), LF power, DC, and AC were inversely associated with UPDRSIII ($r \leq |0.66|$). However, no associations with UPDRSIII were observed in LRRK2-PD. Among the LRRK2-PD patients, DC, AC and HF power were inversely associated with the SCOPA-AUT total score ($r \leq |0.73|$). The HRV measures DC and AC were both highly correlated with rMSSD and HF power ($r \leq 0.92$), and thus considered beat-to-beat HRV measures. Among the healthy controls, age was weakly correlated with most HRV features but not correlated with RE. RE and PE features provided additional information on HRV characteristics as they were weakly or not at all correlated with other HRV measures. PE features and the ordinal pattern statistics that best distinguished LRRK2-PD from controls showed no dependence on HR.

HRV in LRRK2-PD *vs.* controls

Generally, HRV values were greater in LRRK2-PD patients than in controls, although only the beat-to-beat measures of HRV, *i.e.*, rMSSD, SD1, HF, DC, and AC, reached statistical significance (Table 2, Supplementary Fig. 2). Consistent with this, a significant increase in the irregularity of HR dynamics was verified in LRRK2-PD patients, as assessed through RE features (Table 2). DC and RE revealed that 7% and 28% of LRRK2-PD patients (N=1 and N=4, respectively) had standardized values outside the normal range (3–7, mean±2SD). PE and ordinal pattern analysis also revealed an increased irregularity and altered ordinal structure of HR dynamics in the LRRK2-PD patients, which were statistically significant before correcting for multiple comparisons (*e.g.*, $p=0.031$ and $p=0.003$, respectively). The combination of ordinal pattern statistics, which were poorly correlated and distinguished LRRK2-PD from controls, facilitated the identification of cardiac rhythm alterations at an individual level (Fig. 1). The best classification functions performed with an overall accuracy, sensitivity and specificity of 93% each (Supplementary Table 1).

HRV in LRRK2-PD *vs.* iPD

Most of the global HRV measures, LF power, and the beat-to-beat HRV measures DC and AC were significantly greater in LRRK2-PD compared to iPD, although the greatest group differences were seen in central moments and RE features (Table 2). Bradycardia (HR<60 bpm) associated with elevated deceleration capacity (DC>5.5) was found in 21% of LRRK2-PD patients (N=3), compared to 4% of controls (N=1) and 5% of iPD patients (N=1) ($p>0.05$ in both cases). A pattern of periodic HR accelerations between periods of respiratory sinus arrhythmia (Fig. 2) was also found in 21% of LRRK2-PD patients (N=3), compared to 4% of controls (N=1) and 5% of iPD patients (N=1) ($p>0.05$ in both cases).

HRV in LRRK2-NMC *vs.* controls

Overall, HRV values in the LRRK2-NMC group were intermediate between those in controls and LRRK2-PD patients, and no significant differences were found between LRRK2-NMC, RNC and controls. However, there was an increase in the proportion of LRRK2-NMC individuals with values of beat-to-beat HRV measures above the mean standardized interval (4.5–5.5), as was found in the LRRK2-PD group (Fig. 3). Significant differences in the distribution of these features between LRRK2-NMC and controls were found only for HF power ($p<0.05$). By analyzing the HRV Z-scores above the normal range in LRRK2-NMC, it was possible to identify an individual who satisfied criteria for prodromal LRRK2-PD according to the International Parkinson and Movement Disorder Society (see Electronic Supplementary Material) (red bars in Fig. 3). However, not all of the LRRK2-NMC individuals showed high values and a small percentage had values below the normal range for rMSSD and DC (purple bars in Fig. 3).

The irregularity of HR dynamics as quantified by RE was found to be decreased in LRRK2-NMC compared to RNC, before correcting for multiple comparisons (Supplementary Table 2)**.** However, the RE feature H_R that best distinguished LRRK2-PD from controls, $H_R(-\alpha,8)$ as seen in Table 2, showed a higher proportion of values on both sides of its distribution in LRRK2-NMC compared to controls, a pattern similar to that found for DC (Fig. 3). The subgroup of LRRK2-NMC showing the highest DC and the lowest H_R values (28%, N=7) overlapped with the individuals in the LRRK2-PD group (Fig. 4). Among HRV features, central moments revealed the greatest differences in cardiac chronotropism among LRRK2-NMC and RNC or LRRK2-PD (Supplementary Table 2).

Discussion

In this study, we assessed cardiac autonomic modulation in carriers of the *LRRK2* G2019S mutation manifesting and non-manifesting PD, through the HRV analysis of short-term heartbeat interval sequences derived from EKGs recorded in a supine position. Our findings indicated an altered autonomic modulation of cardiac chronotropy early in LRRK2-PD, as suggested by consistent results obtained in both LRRK2-NMC and LRRK2-PD groups. These alterations were different from the cardiac autonomic impairment described for iPD.

We found a significant increase in rMSSD and HF power in LRRK2-PD patients compared to controls, which might suggest an overactive vagal system (Stein et al., 2005). These results are consistent with the findings of a previous study, where a significant increase in both diurnal and nocturnal HF power was reported in a cohort of eight Sardinian LRRK2-PD patients (Solla, 2013). Yet, in previous work reporting on a partially overlapping sample, we found no significant differences in rMSSD or HF power when comparing 20 LRRK2-PD patients with controls (Visanji et al., 2017), although mean values for these measures were greater than controls in 10 LRRK2-PD patients (Goldman et al., 2014). Some of us recently described two distinct clinical-pathological subtypes of G2019S-associated PD, one with typical Lewy pathology and the other devoid of this brain synucleinopathy (Kalia et al., 2015). The latter patients also had evidence for less severe ANS dysfunction. Consistent with this earlier finding, we now report that among LRRK2-PD patients, a higher prevalence of autonomic symptoms is associated with lower markers of cardiac vagal activity. Hence, discrepancies across the HRV findings from LRRK2-PD studies could reflect the neuropathologic heterogeneity of G2019S-associated PD.

We extended our previous findings by integrating novel approaches to HRV analysis. DC and RE were both significantly increased in the LRRK2-PD group compared to controls. In fact, both measures in combination facilitated the identification of five LRRK2-PD patients with abnormally high values of beat-to-beat variability and irregularity of HR. Furthermore, DC and RE values tended to cluster towards both sides of their distribution in LRRK2-NMC, consistent with the existence of LRRK2-NMC subgroups as previously suggested (Dzamko et al., 2016). Since LRRK2-PD is characterized by incomplete penetrance, the LRRK2-NMC subgroup showing a higher DC and irregularity of HR might represent those in a preclinical stage and thus at greater risk of developing PD, as was seen for the prodromal subject. DC has previously been shown to identify patients at higher risk of mortality following

myocardial infarction (Bauer et al., 2006). Although this hypothesis needs testing in longitudinal studies, results suggest that DC and RE are promising biomarkers that could provide prognostic information in LRRK2-NMC, potentially adding to the list of clinical conditions in which these features have proven useful (Bauer et al., 2006; Cornforth et al., 2014).

A further novel interpretation is a differential involvement of the cholinergic and noradrenergic systems in LRRK2-PD and iPD. The novel HRV measures of vagal modulation, DC and AC, were both elevated in LRRK2-PD compared to iPD and controls; whereas LF power, which reflects both vagal and sympathetic contributions to heart rate modulation, was similar in LRRK2-PD compared to controls, but greater compared to iPD. These autonomic alterations were associated with a greater global HRV and HR irregularity in LRRK2-PD compared to iPD, further suggesting pathophysiological differences for the development of cardiac autonomic neuropathy between the two types of PD. Postganglionic noradrenergic lesions are the main cause of cardiac dysautonomia in iPD (Goldstein, 2003), whereas impairment of central vagal feedback loops may account for the cardiac chronotropic alterations found in LRRK2-PD. However, cardiac sympathetic denervation has also been reported in LRRK2-PD (Goldstein et al., 2007). Consistent with our results, decreased HRV and sympathetic involvement have both been found in iPD compared with LRRK2-PD (Tijero et al., 2013; Visanji et al., 2017). Furthermore, increased cholinergic activity was recently reported in the brain of 14 LRRK2-PD and 16 LRRK2-NMC individuals using positron emission tomography (Liu et al.).

Animal studies have provided evidence for pro-inflammatory cytokine activation of vagal afferent signaling, which leads to excitatory synaptic transmission in the *nucleus tractus solitarius* and subsequent synaptic activation of efferent vagal pathways originating in the *nucleus ambiguus* (Watkins et al., 1995), the main source of preganglionic parasympathetic cardiac motoneurons (Geis & Wurster, 1980). Elevated peripheral pro-inflammatory markers have been reported in *LRRK2* G2019S mutation carriers (Brockmann et al., 2016; Dzamko et al., 2016), whereas a central microglial pro-inflammatory response has been associated as well with *LRRK2* mutations (Berg et al., 2015). Previous studies have shown involvement of the vagus nerve in attenuating release of cytokines and downregulating systemic tumor necrosis factor production, providing evidence for a cholinergic anti-inflammatory pathway (Borovikova et al., 2000). Increased peripheral cholinergic drive, as was observed in LRRK2-NMC and LRRK2-PD individuals, might therefore represent an early and sustained compensatory mechanism to counter-balance the inflammation reported in these cohorts.

In summary, our findings are consistent with the results of previous work reporting i) greater central cholinergic activity in *LRRK2* carriers both manifesting and non-manifesting PD (Liu et al.), and ii) increased cardiac cholinergic activity in PD patients with the *LRRK2* mutation (Solla, 2013). Further study to clarify whether this central and peripheral hypercholinergic activity is a G2019S mutation-related mechanism, which operates as a form of prodromal compensation for *LRRK2* immune activation and persists after PD becomes manifest, needs to be addressed.

Limitations

The study patients were under L-dopa treatment, which may have affected autonomic regulation, although previous work have found no significant differences in cardiovascular autonomic function between drug-naïve and dopaminergic drug treated iPD patients (Kim et al., 2016; Turkka et al., 1987). Although HRV differences between groups were consistent, larger sample sizes are required to further explore the heterogeneous presentation of PD. Additional information might also be gained by using 24-hour Holter monitoring.

Conclusions

Our findings extend current knowledge of differences in the non-motor profile of LRRK2-PD and iPD. The *LRRK2* G2019S mutation was found to be associated with a significantly increased beat-to-beat HRV, presumably of cardiac cholinergic origin, suggesting that modifications of central vagal feedback loops might occur in the preclinical, prodromal and clinical stages of LRRK2-PD. Cardiac chronotropic alterations distinguished LRRK2-PD from

iPD patients, supporting distinct pathological mechanisms underlying both PD types. Our results raise the possibility that Rényi entropy and HRV measures of vagal modulation may be relevant biomarkers of prodromal LRRK2-PD. Further research and longitudinal studies, aimed at performing an integral evaluation of cardiovascular autonomic function in different stages of LRRK2-PD, are needed to understand the full clinical importance of our findings.

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Author contributions

CM, NV, and AL had the conception of and designed the study; CM, NV, BS, and SG organized the research project; NV, BS, and SG contributed to data collection; CC, CM, NV, DC, LS, ME, PS, HJ, and AM contributed to data analysis and interpretation; CC drafted the manuscript; CM, PS, AL, and HJ contributed to writing and critical review of the manuscript; NV, DC, LS, BS, SG, ME and AM contributed to manuscript revision. All authors read and approved the final version for publication.

Conflict of interest

CCN reports employment with the University of Havana, and grants from the International Brain Research Organization, International Parkinson and Movement Disorder Society, and the Free University of Brussels, Belgium.
CM reports consultancies with Acorda Therapeutics; honoraria for teaching from EMD Serono, steering committee for Michael J Fox Foundation; grants from the Michael J Fox Foundation, Canadian Institutes of Health Research, International Parkinson and Movement Disorder Society, and National Institutes of Health Research, and employment with University Health Network.
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DJC reports none.
LS reports none.
BS reports none.
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ME reports none.
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HFJ reports none.
AM reports employment with the University of Havana.

References

Bandt, C., & Pompe, B. (2002). Permutation entropy: a natural complexity measure for time series. *Phys Rev Lett, 88*(17), 174102. doi: 10.1103/PhysRevLett.88.174102

Bauer, A., Kantelhardt, J. W., Barthel, P., Schneider, R., Mäkikallio, T., Ulm, K., . . . Georg, S. (2006). Deceleration capacity of heart rate as a predictor of mortality after myocardial infarction: cohort study. *Lancet, 367*, 1674–1681.

Berg, D., Postuma, R. B., Adler, C. H., Bloem, B. R., Chan, P., Dubois, B., . . . Deuschl, G. (2015). MDS research criteria for prodromal Parkinson's disease. *Movement disorders : official journal of the Movement Disorder Society, 30*(12), 1600-1611. doi: 10.1186/s12883-015-0491-1

10.1002/mds.26431

Borovikova, L. V., Ivanova, S., Zhang, M., Yang, H., Botchkina, G. I., Watkins, L. R., . . . Tracey, K. J. (2000). Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature, 405*(6785), 458-462. doi: 10.1038/35013070

Brockmann, K., Apel, A., Schulte, C., Schneiderhan-Marra, N., Pont-Sunyer, C., Vilas, D., . . . Maetzler, W. (2016). Inflammatory profile in LRRK2-associated prodromal and clinical PD. *J Neuroinflammation, 13*(1), 122. doi: 10.1186/s12974-016-0588-5

Cornforth, D. J., Tarvainen, M. P., & Jelinek, H. F. (2014). How to Calculate Renyi Entropy from Heart Rate Variability, and Why it Matters for Detecting Cardiac Autonomic Neuropathy. *Front Bioeng Biotechnol, 2*, 34. doi: 10.3389/fbioe.2014.00034

Dzamko, N., Rowe, D. B., & Halliday, G. M. (2016). Increased peripheral inflammation in asymptomatic leucine-rich repeat kinase 2 mutation carriers. *Movement disorders : official journal of the Movement Disorder Society, 31*(6), 889-897. doi: 10.1002/mds.26529

Geis, G. S., & Wurster, R. D. (1980). Cardiac responses during stimulation of the dorsal motor nucleus and nucleus ambiguus in the cat. *Circulation Research, 46*(5), 606-611. doi: 10.1161/01.res.46.5.606

Goldman, S., Schuele, B., Bhudhikanok, G., Cash, S., Korell, M., Amiri, Y., . . . Tanner, C. (2014). Heart Rate Variability in LRRK2 Parkinson’s Disease (S37.004). *Neurology, 82*(10 Supplement).

Goldstein, D. S. (2003). Dysautonomia in Parkinson's disease: neurocardiological abnormalities. *Lancet Neurol, 2*(11), 669-676.

Goldstein, D. S., Imrich, R., Peckham, E., Holmes, C., Lopez, G., Crews, C., . . . Hallett, M. (2007). Neurocirculatory and nigrostriatal abnormalities in Parkinson disease from LRRK2 mutation. *Neurology, 69*(16), 1580-1584. doi: 10.1212/01.wnl.0000268696.57912.64

Healy, D. G., Falchi, M., O'Sullivan, S. S., Bonifati, V., Durr, A., Bressman, S., . . . Wood, N. W. (2008). Phenotype, genotype, and worldwide genetic penetrance of LRRK2-associated Parkinson's disease: a case-control study. *Lancet Neurol, 7*(7), 583-590. doi: S1474-4422(08)70117-0 [pii]

10.1016/S1474-4422(08)70117-0

Hughes, A. J., Daniel, S. E., Kilford, L., & Lees, A. J. (1992). Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J Neurol Neurosurg Psychiatry, 55*(3), 181-184.

Kalia, L. V., & Lang, A. E. (2015). Parkinson's disease. *The Lancet, 386*(9996), 896-912. doi: 10.1016/S0140-6736(14)61393-3

Kalia, L. V., Lang, A. E., Hazrati, L. N., Fujioka, S., Wszolek, Z. K., Dickson, D. W., . . . Marras, C. (2015). Clinical correlations with Lewy body pathology in LRRK2-related Parkinson disease. *JAMA Neurol, 72*(1), 100-105. doi: 1934714 [pii]

10.1001/jamaneurol.2014.2704

Kallio, M., Haapaniemi, T., Turkka, J., Suominen, K., Tolonen, U., Sotaniemi, K., . . . Myllyla, V. (2000). Heart rate variability in patients with untreated Parkinson's disease. *Eur J Neurol, 7*(6), 667-672. doi: ene127 [pii]

Kim, J.-S., Lee, S.-H., Oh, Y.-S., Park, J.-W., An, J.-Y., Park, S.-K., . . . Lee, K.-S. (2016). Cardiovascular Autonomic Dysfunction in Mild and Advanced Parkinson's Disease. *Journal of Movement Disorders, 9*(2), 97-103. doi: 10.14802/jmd.16001

Liu, S.-Y., Wile, D. J., Fu, J. F., Valerio, J., Shahinfard, E., McCormick, S., . . . Stoessl, A. J. The effect of LRRK2 mutations on the cholinergic system in manifest and premanifest stages of Parkinson's disease: a cross-sectional PET study. *The Lancet Neurology, 17*(4), 309-316. doi: 10.1016/S1474-4422(18)30032-2

Machado, A., Migliaro, E. R., Contreras, P., & Coro, F. (2000). Automatic filtering of RR intervals for heart rate variability analysis. *A. N. E., 5*(3), 255-261.

Maetzler, W., Karam, M., Berger, M. F., Heger, T., Maetzler, C., Ruediger, H., . . . Berg, D. (2015). Time- and frequency-domain parameters of heart rate variability and sympathetic skin response in Parkinson's disease. *J Neural Transm (Vienna), 122*(3), 419-425. doi: 10.1007/s00702-014-1276-1

Marras, C., Schule, B., Munhoz, R. P., Rogaeva, E., Langston, J. W., Kasten, M., . . . Lang, A. E. (2011). Phenotype in parkinsonian and nonparkinsonian LRRK2 G2019S mutation carriers. *Neurology, 77*(4), 325-333. doi: WNL.0b013e318227042d [pii]

10.1212/WNL.0b013e318227042d

Paisan-Ruiz, C., Lang, A. E., Kawarai, T., Sato, C., Salehi-Rad, S., Fisman, G. K., . . . Rogaeva, E. (2005). LRRK2 gene in Parkinson disease: mutation analysis and case control association study. *Neurology, 65*(5), 696-700. doi: 65/5/696 [pii]

10.1212/01.wnl.0000167552.79769.b3

Rodriguez, M., Sabate, M., & Troncoso, E. (1996). Time and frequency domain analysis for the assessment of heart autonomic control in Parkinson's disease. *J Neural Transm, 103*(4), 447-454.

Solla, P. (2013). *[Non-motor symptoms and cardiovascular dysautonomia in Sardinian patients suffering from Parkinson's disease with and without mutations of the LRRK2 gene].* (Doctoral Thesis Doctoral), Universita' degli Studi di Cagliari. Retrieved from http://veprints.unica.it/877/ UniCA Eprints database.

Stein, P. K., Domitrovich, P. P., Hui, N., Rautaharju, P., & Gottdiener, J. (2005). Sometimes higher heart rate variability is not better heart rate variability: results of graphical and nonlinear analyses. *J Cardiovasc Electrophysiol, 16*(9), 954-959. doi: JCE40788 [pii]

10.1111/j.1540-8167.2005.40788.x

Tijero, B., Gomez Esteban, J. C., Somme, J., Llorens, V., Lezcano, E., Martinez, A., . . . Zarranz, J. J. (2013). Autonomic dysfunction in parkinsonian LRRK2 mutation carriers. *Parkinsonism Relat Disord, 19*(10), 906-909. doi: S1353-8020(13)00195-8 [pii]

10.1016/j.parkreldis.2013.05.008

Turkka, J. T., Tolonen, U., & Myllyla, V. V. (1987). Cardiovascular reflexes in Parkinson's disease. *Eur Neurol, 26*(2), 104-112.

Visanji, N. P., Bhudhikanok, G. S., Mestre, T. A., Ghate, T., Udupa, K., AlDakheel, A., . . . Marras, C. (2017). Heart rate variability in leucine-rich repeat kinase 2-associated Parkinson's disease. *Movement disorders : official journal of the Movement Disorder Society, 32*(4), 610-614. doi: 10.1002/mds.26896

Watkins, L. R., Goehler, L. E., Relton, J. K., Tartaglia, N., Silbert, L., Martin, D., & Maier, S. F. (1995). Blockade of interleukin-1 induced hyperthermia by subdiaphragmatic vagotomy: evidence for vagal mediation of immune-brain communication. *Neurosci Lett, 183*(1-2), 27-31.

West, A. B., Moore, D. J., Biskup, S., Bugayenko, A., Smith, W. W., Ross, C. A., . . . Dawson, T. M. (2005). Parkinson's disease-associated mutations in leucine-rich repeat kinase 2 augment kinase activity. *Proc Natl Acad Sci U S A, 102*(46), 16842-16847. doi: 10.1073/pnas.0507360102

Table 1. Clinical features of participants

Feature	Control	LRRK2-PD	LRRK2-PD *vs.* Control	iPD	LRRK2-PD *vs.* iPD	NMC	NMC *vs.* LRRK2-PD	RNC	NMC *vs.* RNC
N	27	14	-	20	-	25	-	32	-
Fem/Male	15/12	3/11	0.037	10/10	ns	8/17	ns	16/16	ns
Age (yrs)	58.7 ±	63.3 ±	ns	64.1 ±	ns	50.3 ±	0.004	45.2 ±	ns
HR (bpm)	69 ± 11	67 ± 10	ns	69 ± 7	ns	63 ± 9	ns	63 ± 10	ns
DD (yrs)	na	10.8 ± 5.1	-	6.3 ± 6.0	0.015	na	-	na	-
UPDRSIII	1.00 ±	16.70 ±	<0.0001	23.00 ±	ns	2.00 ±	<0.0001	0.00 ±	0.026
SCOPA-AUT	8.53 ±	23.5 ±	0.0003	nd	-	10.07 ±	0.001	8.23 ±	ns

Values are expressed as mean ± standard deviation or number of cases (N). UPDRSIII score is expressed as median ± interquartile range. Sex differences were assessed using a Chi-Square test; mean heart rate (HR) using a multiple regression analysis adjusted for age, sex and the effect of age and sex interaction; age, log-transformed disease duration (DD), and SCOPA-AUT score using a *t*-test; and UPDRSIII using a Mann-Whitney *U* test. DD: disease duration; Fem: female; HR: mean heart rate; iPD: idiopathic Parkinson's disease group; LRRK2-PD: LRRK2-associated Parkinson's disease group; N: number of cases; na: not applicable; nd: not determined; NMC: LRRK2-non-manifesting carriers group; ns: $p \geq 0.05$, not statistically significant; RNC: related non-carriers group; SCOPA-AUT: Scales for Outcomes in Parkinson's Disease-Autonomic; UPDRSIII: Unified Parkinson's Disease Rating Scale part 3.

Table 2. Heart rate variability in Parkinson's disease patients and healthy controls

Description	Feature	Control	LRRK2-PD	LRRK2-PD *vs.* Control	iPD	LRRK2-PD *vs.* iPD
Overall HRV	LogSDNN	3.45 ± 0.46	3.51 ± 0.34	ns	3.19 ± 0.51	**0.005**
	LogCV	1.27 ± 0.42	1.31 ± 0.33	ns	1.03 ± 0.45	**0.005**
	LogASI	3.42 ± 0.91	3.30 ± 0.65	ns	3.89 ± 0.96	0.010
	LogTP	2.44 ± 0.09	2.46 ± 0.07	ns	2.39 ± 0.10	0.047
	H	5.69 ± 0.59	5.81 ± 0.43	ns	5.36 ± 0.65	**0.001**
	LogMom	-1.23 ±	-1.91 ± 1.39	ns	-1.05 ± 1.13	**0.01**
	LogMom	1.25 ± 0.26	1.17 ± 0.33	ns	1.38 ± 0.38	**0.0006**
	LogMom	1.18 ± 1.15	0.06 ± 1.68	ns	1.48 ± 1.12	**0.003**
	LogMom	3.09 ± 0.68	2.77 ± 0.75	ns	3.32 ± 0.88	**0.0004**
	LogMom	3.55 ± 1.33	2.19 ± 1.52	ns	3.87 ± 1.49	**0.0008**
	LogMom	5.19 ± 1.13	4.57 ± 1.19	ns	5.52 ± 1.38	**0.0003**
	LogMom	-5.90 ±	4.41 ± 2.01	ns	6.25 ± 1.96	**0.0007**
Beat-to-beat HRV	LogrMSS	2.92 ± 0.55	3.13 ± 0.40	**0.015**	2.80 ± 0.56	ns
	LogHF	2.29 ± 0.12	2.32 ± 0.09	**0.012**	2.25 ± 0.12	ns
	LogDC	1.98 ± 0.59	2.13 ± 0.47	**0.006**	1.68 ± 0.64	0.026
	Log|AC|	1.97 ± 0.58	2.11 ± 0.42	**0.012**	1.71 ± 0.66	0.032
Intermediate-term HRV	LogLF	2.38 ± 0.10	2.40 ± 0.09	ns	2.32 ± 0.12	0.032
HR Irregularity	$H_R(-\alpha,4)$	1.29 ± 0.04	1.23 ± 0.06/ 1.05 ± 0.02	**0.002**	1.06 ± 0.02	**0.009**
	$H_R(+\alpha,4)$	0.94 ± 0.02	0.95 ± 0.02	**0.004**	0.94 ± 0.02	ns
	$H_R(-\alpha,8)$	1.24 ± 0.05	1.18 ± 0.07/ 1.05 ± 0.04	**0.002**	1.07 ± 0.04	**0.001**
	$H_R(+\alpha,8)$	0.94 ± 0.02	0.96 ± 0.02	**0.003**	0.94 ± 0.03	ns
	$H_R(-\alpha,16)$	1.12 ± 0.06	1.07 ± 0.05/ 1.04 ± 0.03	**0.003**	1.06 ± 0.04	**0.0004**
	$H_R(+\alpha,16)$	0.93 ± 0.02	0.95 ± 0.02/ 0.98 ± 0.01	**0.003**	0.97 ± 0.01	**0.0008**

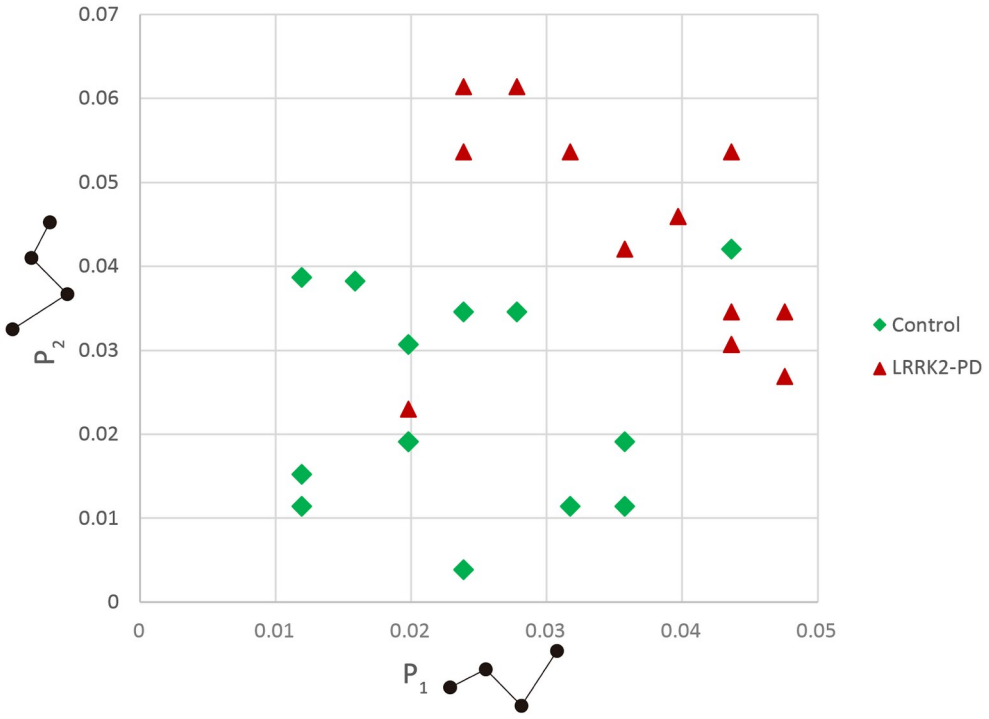
Table 2 shows heart rate variability (HRV) values for the LRRK2-associated Parkinson's disease (LRRK2-PD), idiopathic Parkinson's disease (iPD), and control groups. Values are expressed as mean ± standard deviation. Beat-to-beat HRV features reflect the vagal modulation of heart rate (HR), whereas the remaining features may reflect the contribution of both vagal and sympathetic modulation. Group contrasts show p-values for mean HRV differences as assessed through multiple regression analysis adjusted for age, sex, and mean HR. The LRRK2-PD *vs.* iPD contrasts were also adjusted for disease duration. Only significant values at $p<0.05$ are shown. P-values remaining significant after correcting for multiple comparisons appear in bold text. The best results of Rényi entropy H_R calculated over sequences of length λ = 4, 8, and 16 cardiac interbeat intervals, for positive and negative order α are shown. As distinct α values may be used, the values of the H_R revealing the greatest differences for the contrasts LRRK2-PD *vs.* Control and LRRK2-PD *vs.* iPD are shown in that order. Increased irregularity of HR changes is manifested as an increase in H_R with positive order $+\alpha$ or as a decrease in H_R with negative order $-\alpha$. α: order of Rényi entropy, $\alpha=\{-5,-4,-3,-2,-1,+1,+2,+3,+4,+5\}$; AC: acceleration capacity of heart rate; ASI: autonomic stress index; CV: coefficient of variation of normal-to-normal intervals; DC: deceleration capacity of heart rate; H: Shannon entropy; H_R: Rényi entropy; HF: power spectral density of the high frequency band (0.15–0.4 Hz); LF: power spectral density of the low frequency band (0.04–0.15 Hz); Log: log-transformed value; Mom3-Mom9: standardized central moments of heartbeat interval distribution of 3rd to 9th order; ns: $p\geq0.05$, not statistically significant; rMSSD: square root of the mean of the sum of the squares of differences between adjacent normal-to-normal intervals; SDNN: standard deviation of normal-to-normal intervals; TP: power spectral density of the total power band (0.04–0.4 Hz).

Fig. 1 Discrimination of LRRK2-associated Parkinson's disease (LRRK2-PD) patients and healthy controls based on ordinal pattern statistics of heart rate. The discrimination of patients and controls based on the probabilities of ordinal pattern P1 and P2 achieved the best classification accuracy (discriminant model $p<0.0001$). P1 and P2 were calculated for patterns expanding four heartbeat intervals over the time scales 13 and 16, respectively.

Fig. 2 Periodic heart rate accelerations in LRRK2-associated Parkinson's disease (LRRK2-PD). A, Tachogram of a LRRK2-PD patient showing heart rate accelerations (indicated by blue arrows) separated by periods of respiratory sinus arrhythmia (one of these periods is illustrated by the blue tracing). B, Tachogram of a control participant comparable for age, sex, and mean heart rate to the patient in panel A. NN intervals are plotted *vs.* the interval order (horizontal axis). NN: normal-to-normal cardiac interbeat interval.

Fig. 3 Standardized distribution of beat-to-beat variability and irregularity measures of heart rate dynamics in LRRK2-non-manifesting carriers (LRRK2-NMC). Standardized distributions. Compared to controls, the mean interval (green bar) for rMSSD, HF, and AC is shortened and shifted to the left in the LRRK2-NMC and LRRK2-PD groups, indicating a greater proportion of values above the mean. For DC and H_R, the mean interval in the LRRK2-NMC group is only shortened, indicating a greater proportion of values below and above the mean. AC: acceleration capacity of heart rate; DC: deceleration capacity of heart rate; H_R: Rényi entropy; HF: power spectral density of the high frequency band (0.15–0.4 Hz); rMSSD: square root of the mean of the sum of the squares of differences between adjacent normal-to-normal intervals.

Fig. 4 Subgroup of LRRK2-non-manifesting carriers (LRRK2-NMC) overlapping with the LRRK2-associated Parkinson's disease (LRRK2-PD) group. The subgroup of LRRK2-NMC with the highest DC and the lowest H_R overlaps with the LRRK2-PD group. DC: deceleration capacity of heart rate; H_R: Rényi entropy.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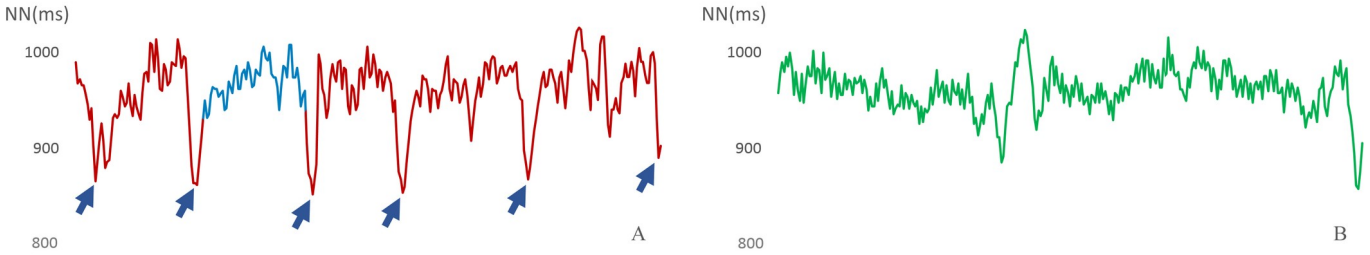
524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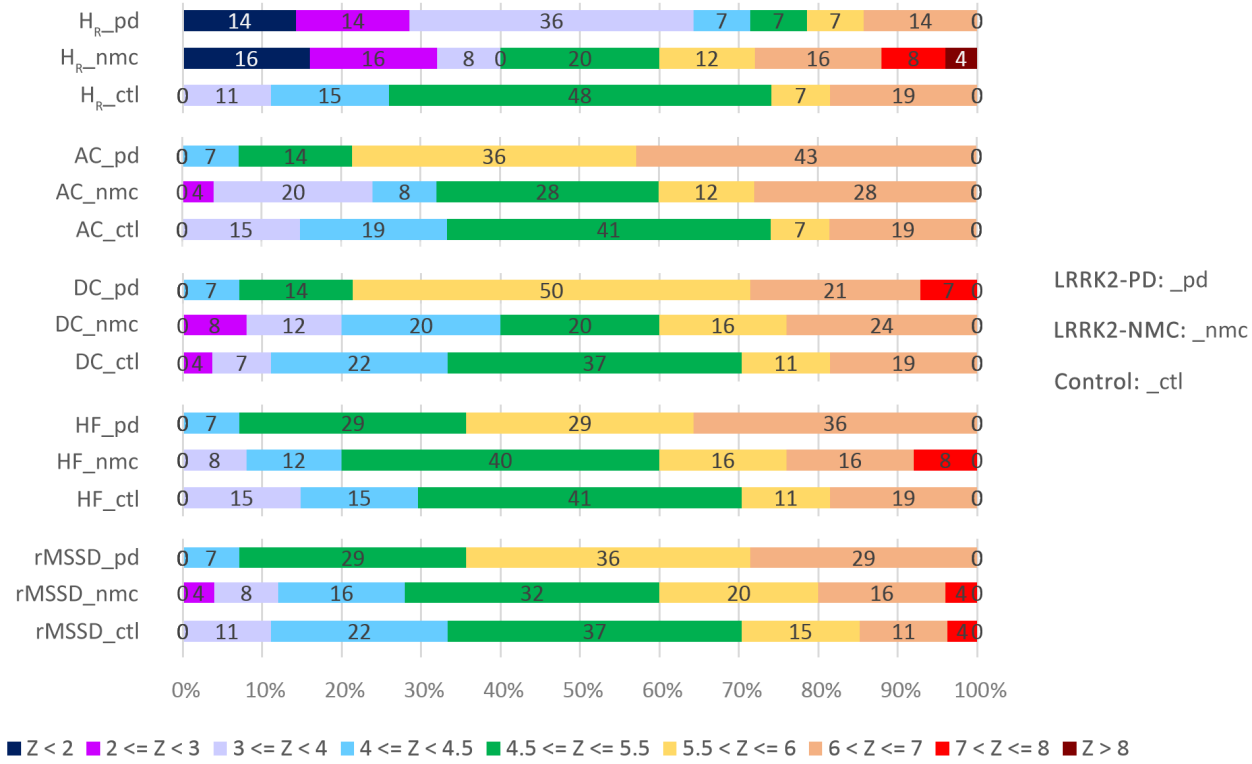
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AC_ctl 0 15 19 41 7 19 0
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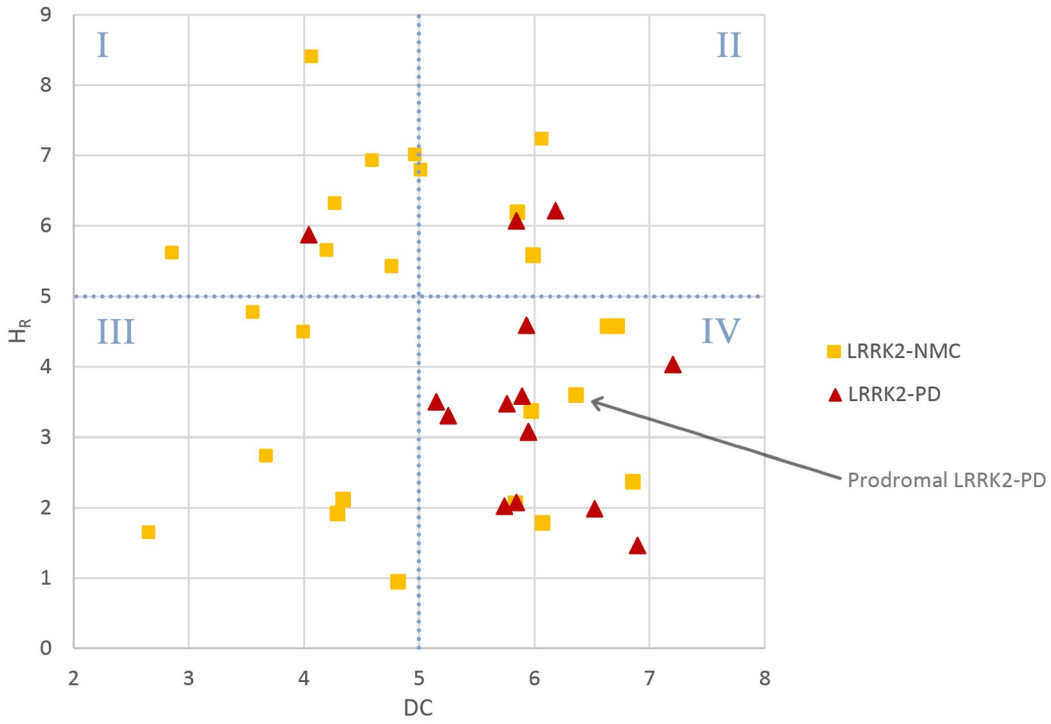
HF_pd 0 7 29 29 36 0
HF_nmc 0 8 12 40 16 16 8 0
HF_ctl 0 15 15 41 11 19 0
rMSSD_pd 0 7 29 36 29 0
rMSSD_nmc 0 4 8 16 32 20 16 4 0
rMSSD_ctl 0 11 22 37 15 11 4 0
0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%
LRRK2-PD: _pd
LRRK2-NMC: _nmc
Control: _ctl
Z < 2
2 <= Z < 3
3 <= Z < 4
4 <= Z < 4.5
4.5 <= Z <= 5.5
5.5 < Z <= 6
6 < Z <= 7
7 < Z <= 8
Z > 8

526


I
II
III
IV
H_R
DC
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2 3 4 5 6 7 8
LRRK2-NMC
LRRK2-PD
Prodromal LRRK2-PD

527